Title
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Monitored changes in the number of copies of a gene during DNA replication control the timing of sporulation in bacteria. This discovery links monitoring of replication to the concept that a gene's location on a chromosome can influence cell traits.

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For decades, it has been known that the location of a gene on its chromosome can influence the level at which it is expressed[1]. Most bacterial chromosomes are circular, and their replication begins at a single bi-directional origin. As such, during chromosome replication, genes close to the origin of replication will transiently be present in more copies (present at a higher dosage) than genes close to the terminus of replication. Altering the distance of a gene from the origin of replication systematically alters its level of expression during the cell's replication cycle[2–4]. But until now, the significance of gene location has largely focused on whether highly expressed genes are preferentially located in the origin-proximal half of the chromosome, because this provides the cell with a growth advantage, owing to a positive gene-dosage effect[5]. Writing in *Cell*, Narula *et al.*[6] report a new twist on the role of chromosomal location in gene function, in coordinating sporulation with chromosome replication in the bacterium *Bacillus subtilis*.

When subject to starvation, *B. subtilis* can initiate a cascade of protein phosphorylation that leads to sporulation, producing a dormant spore that is resistant to starvation conditions and that can eventually resume growth under favorable conditions. The first components of this phosphorelay are a kinase enzyme called KinA and a response-regulator protein, Spo0F. Although phosphorylation of Spo0F by KinA is necessary for the activation of early sporulation genes, if Spo0F is highly expressed it can also inhibit the activity of KinA[6-7], thus inducing a negative feedback loop that inhibits the phosphorelay .

Intriguingly, the *spo0F* gene is located near the origin of replication, whereas the *kinA* gene is located close to the replication terminus. And indeed, Narula *et al.* report that the positions of *spo0F* and *kinA* seem to be crucial for their ability to efficiently regulate

sporulation. Because *spo0F* is located close to the replication origin, during replication there is a transient two-fold increase in its dosage relative to *kinA*. By using computer simulations and then verifying their models *in vivo*, the authors showed that the transient increase in Spo0F concentration inhibits KinA until replication is completed, leading to pulsing dynamics of sporulation-gene expression during each cell cycle (Fig. 1). Cells will only cross the threshold of sporulation-gene expression to initiate differentiation after a sufficient level of KinA concentration is achieved through a positive-feedback loop, which take several rounds of cell division to acheive[9].

Narula and colleagues then performed translocation experiments, in which they moved *spo0F* or *kinA* towards the terminus or origin of replication respectively. These translocations abolished pulsing, confirming that a transient imbalance in the dosage of the two genes is necessary for pulsing of early sporulation-gene expression and for proper coordination of the sporulation program with DNA replication. These data, together with the authors' finding that the relative locations of *kinA* and *spo0F* are similar in 45 other species of sporulating bacteria, show for the first time that cells might have evolved to put interacting genes at different locations on the chromosome, to control how the genes' products function.

Monitoring chromosome replication status is crucial for many species. In the case of *B. subtilis*, initiation of sporulation without complete chromosomes for both the mother cell and the future spore cell would be a waste of resources. It has long been known[7] that a checkpoint is activated to inhibit sporulation when DNA is damaged or replication is defective. Narula and colleagues' study has identified a remarkably simple mechanism by which cells can monitor the replication status of the chromosome.

The regulatory mechanism presented in this study deepens our understanding of the potential variety of mechanisms that might regulate cellular trait changes. But the study also raises several interesting avenues for further investigation. For example, it is unclear whether this particular situation is a biological one-off. It seems more likely that there are other traits, both in *B. subtilis* and in other organisms, that are regulated by temporal variations in gene-product ratios associated with gene location.

It also remains to be seen whether more-complex versions of this mechanism exist, involving more than two genes, and whether such mechanisms could be involved in replication fidelity. For instance, could this type of regulatory mechanism act as a brake on chromosomal rearrangements such as inversions, which might disrupt the relative locations of genes in regulatory networks that rely on dosage imbalances? This work illustrates the potential importance of gene location in chromosomes. It will motivate future experiments in chromosome remodeling, and perhaps also a re-examination of old data to assess whether arbitrary choices made in genetic engineering might have affected experimental outcomes.

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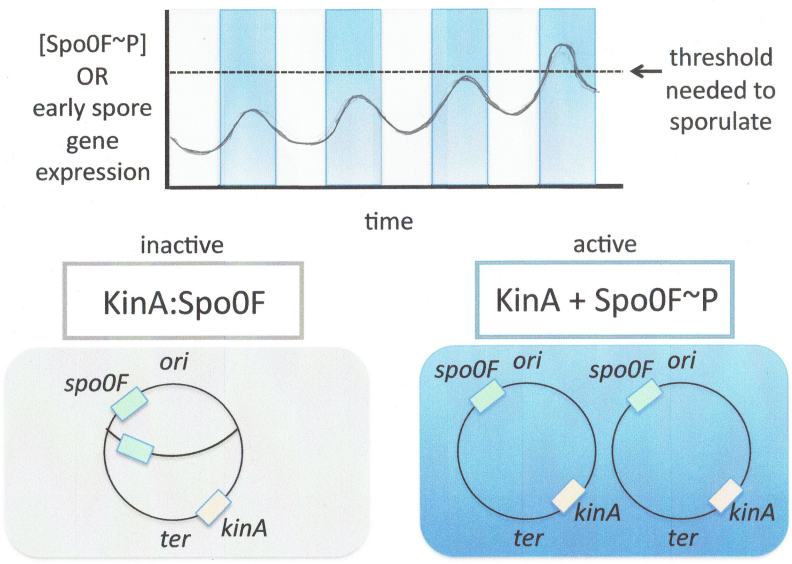

Figure 1 | A genetic imbalance regulates sporulation. The bacterium *Bacillus subtilis* sporulates by activating a phosphorylation cascade, which begins with phosphorylation of the protein Spo0F by the enzyme KinA. **a**, Narula *et al.* report that the *spo0F* gene is located close to the site at which DNA replication originates on the *B. subtilis* chromosome,

whereas *kinA* is close to the replication terminus. During chromosome replication, the concentration of Spo0F in the cell transiently increases relative to the level of KinA, owing to a difference in gene copy number. This activates a negative-feedback pathway that inactivates sporulation genes. Once replication is completed, the disparity is resolved, and sporulation-gene-expression pathways are activated. **b**, As such, sporulation genes are activated in pulses during sequential cell cycles. Blue indicates times when the chromosome is fully replicated, grey indicates partial replication. Once a threshold level of expression is reached, sporulation occurs.